Executive Summary (4th DSRC)

Name of Research Scholar: Pankaj Kumar **Name of Supervisor:** Prof. Geeta Aggarwal

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Title: Development and evaluation of nanostructured lipid carrier for targeted delivery of gefitinib in breast cancer

Abstract (500-800 words)

Introduction: Breast cancer is the most frequently occurring cancer in women and stands as the second-leading cause of female mortality. Gefitinib is tyrosine kinase inhibitors (TKIs) which inhibits the activity of the epidermal growth factor receptor (EGFR) in breast cancer. Nonetheless, the application of gefitinib is limited due to its low water solubility, stability, and low oral bioavailability resulting from extensive first-pass metabolism and p-gp efflux in the gastrointestinal tract. Lipid-based nanocarriers show promise in the emerging realm of oral drug delivery for both hydrophilic and lipophilic drugs. They serve as an alternative to conventional colloidal drug delivery systems. Nano lipid carrier system (NLCs) helps to overcome the biopharmaceuticals and toxicological challenges associated with gefitinib. **Objectives:** The objective of this study is to develop an effective oral lymphatic targeting lipid based carrier system of gefitinib for the treatment of breast cancer and to overcome the issues associated with conventional drug delivery. **Methodology**: Gefitinib loaded Nanaolipid carrier system (GTB-NLCs) was prepared by hot emulsification ultrasonication technique. Stearic acid and oleic acid were selected as the solid and liquid lipids, respectively, while poloxamer 188 and Tween 80 were chosen as the surfactants. A binary mixture consisting of 70:30 ratio was selected. The formulation parameters were optimized using Box-Behnken design method. Subsequently, in vitro characterization i.e. particle size, PDI, zeta potential, surface morphology (SEM & TEM), solid-state characteristics (DSC & XRD), entrapment efficiency (%EE) and *in vitro* drug release studies were conducted. Furthermore, in vitro intracellular uptake and cell cytotoxicity assay was performed on MCF-7 cell line. Fluorescence depth permeation study was also performed through CLSM. *In vivo* pharmacokinetics, pharmacodynamic and acute toxicity studies were performed on female wistar rats to establish the proof of concept. **Results**: The particle size, PDI and zeta potential of the optimized GTB-NLCs was found to be 165.2 ± 3.17 nm, 0.262 ± 0.19 and -24.4 ± 5.25 mV. Morphological observations showed spherical particles with smooth surfaces and no signs of breakage. Solid state characterization studies confirmed the complete encapsulation of the GTB within the lipid matrix, causing a transition from crystalline to amorphous state. The entrapment efficiency percentage of GTB-loaded NLCs was calculated to be 91.2 ± 2.1%. In the drug release study, free GTB released 30% and 9% from plain GTB and GTB-NLCs in 2 hr at pH 1.2. At pH 7.2, GTB-NLCs released 73%, compared to 33% from plain for 24 hrs indicating sustained release. Stability studies confirmed prepared formulation was stable for three months under various temperature conditions. GTB-NLCs showed higher cell cytotoxicity as compared to their GTB with IC50 134.3 and 69.12 µg/mL of plain GTB and GTB-NLCs. The GTB-NLCs did not show any toxicity on vital organs such as liver, kidney, heart, and spleen whereas rats treated with plain GTB showed toxicity on organs hence, it prevented the toxicity associated with plain GTB. The apparent permeability coefficient (Papp) of GTB from NLCs (2.32×10^{4} cm/min) was significantly higher than plain GTB

(0.46×10^4 cm/min) respectively. Fluorescence depth permeation study revealed enhanced intestinal permeation with the NLCs formulation (167.6 µm) compared to the plain ROD-B solution (88.1 µm). Pharmacokinetic study showed higher relative oral bioavailability of GTB from GTB-NLCs was 3.62-fold higher as compared with plain GTB. Pharmacodynamics study showed that GTB-NLCs suppressed the tumor growth in comparison with GTB suspension. GTB-NLCs reduce the level of cancer antigen (CA-15-3) and EGFR protein in blood. **Conclusion**: All the objectives of the present work were successfully achieved. GTB into NLCs holds a promising approach to enhance the oral bioavailability and prevent organ toxicities of GTB. The study findings offered novel insights into effective delivery methods in cancer treatment. **Outcome:** This study has the potential to be a significant advancement in the development of lipid-based carrier system for GTB, leading to enhanced anti-cancer efficacy in the treatment of EGFR-driven breast cancer.

	Recommendation of 4th DSRC	**Compliance of 4th DSRC**
1.	The work done by the candidate was approved by the DSRC committee.	The compilation of the research work is compiled. Patent is filed.
2.	It was recommended to compile the work in the thesis format for the pre-submission.	
3.	It was also suggested that work may be carried forward by the next student for stability studies and process of technology transfer for the formulation may be initiated.	

	Recommendation of 3rd URAC	**Compliance of 3rd URAC**
1.	The publication and conclusion should be written in proper manner.	The conclusion and conclusion are written in proper manner.

List of publications

1. **Kumar, P.,** Mangla, B., Javed, S., Ahsan, W., & Aggarwal, G. (2023). Amelioration of the therapeutic potential of gefitinib against breast cancer using nanostructured lipid-based carriers. *Nanomedicine.* Print ahead (Research article)
2. **Kumar, P.,** Mangla, B., Beg, S., Afzal, O., Altamimi, A. S. A., Almalki, W. H., ... & Aggarwal, G. (2022). Optimization and validation of stability indicating RP-HPLC method for the quantification of gefitinib in bulk drug and nanoformulations: An application towards in vitro and ex vivo performance evaluation. *Arabian Journal of Chemistry*, *15*(12), 104333. (Research article)

3. **Kumar, P.,** Mangla, B., Javed, S., Ahsan, W., Musyuni, P., Sivadasan, D., & Aggarwal, G. (2023). A review of nanomaterials from synthetic and natural molecules for prospective breast cancer nanotherapy. *Frontiers in Pharmacology*, *14*, 1149554. (Review article)
4. **Kumar, P.,** Mangla, B., Javed, S., Ahsan, W., Musyuni, P., Ahsan, A., & Aggarwal, G. (2023). Gefitinib: an updated review of its role in the cancer management, its nanotechnological interventions, recent patents and clinical trials. *Recent Patents on Anti-Cancer Drug Discovery*, *18*(4), 448-469. (Review article)

List of conferences

1. Presented a paper entitled "Gefitinib lipid-based nanoformulation improves oral bioavailability and reduces toxicities: formulation optimization, ex vivo assessment, and efficacy against breast cancer treatment" at the **International Conference on Nanotechnology and Drug Delivery (ICNDD-23) held at Jamia Hamdard in New Delhi on February 9-10, 2023."**
2. Presented a paper entitled "Development of a Nano Lipid Carrier System of Gefitinib for the Treatment of Breast Cance" at the **National Conference on Opportunities for MSMEs from Drug Discovery to Formulation Development held at DPSRU in New Delhi from January 11-14, 2023."**

Attachments

Plagiarism report (Turnitin): Attached

List of Publications (first page): Attached as annexure I

List of conferences attended (proof): Attached as annexure II

Patents Published / granted (proof): Attached as annexure III

Signature of student

Signature of supervisor